

Antioxidant protection enzyme activity in the blood serum and large intestinal mucosa of rats with prolonged gastric hypochlorhydria and given multiprobitotics

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The activity of antioxidant protection enzymes in the blood serum and colon mucosa in rats was studied under the conditions of 28-days administration of omeprazole on its own and omeprazole together with multiprobitotics "Symbiter" and "Apibact". Physiological and biochemical study methods were applied. It was found that after omeprazole administration, the activity of superoxide dismutase in the blood serum decreased, and the activity of catalase increased compared to the control. With the co-administration of omeprazole and multiprobitotics, the activity of superoxide dismutase increased compared to the group of rats that received omeprazole only during the same time, but remained less compared to the control group. The content of reduced glutathione in the blood serum of rats after administration of omeprazole decreased, the activity of glutathione peroxidase and glutathione transferase increased, and the activity of glutathione reductase decreased compared to the control. With co-administration of omeprazole and multiprobitotics, the serum RG content was at the control level, the activity of glutathione reductase exceeded the control values. The activity of glutathione reductase decreased compared to the group receiving omeprazole only. The activity of glutathione reductase increased and did not differ from the control values. In the colon mucosa, superoxide dismutase and catalase activity decreased compared to control. With the combined administration of omeprazole and multiprobitotics, superoxide dismutase and catalase activity increased and even exceeded the control values. With the administration of omeprazole, the reduced glutathione content in the colon mucosa was lower than that in the control. The activity of glutathione peroxidase increased and glutathione transferase and activity of glutathione reductase decreased compared to the control. With co-administration of omeprazole and multiprobitotics to rats, the reduced glutathione content increased compared to the group of rats administered omeprazole only, and even exceeded that in the control.

Keywords: omeprazole; superoxide dismutase; catalase; glutathione; antioxidant protection enzymes.

Introduction

Today, an important problem for research remains the functioning of the digestive system in the conditions of prolonged gastric hypochlorhydria, which leads to hypergastrinemia, which in its turn is known to be a significant factor in the growth and development of tumours in the gastrointestinal tract (Burkitt et al., 2009). Most frequently carcinoid tumours of the stomach develop in patients in whom diseases are associated with hypergastrinemia (Stepanov et al., 2000). These are diseases such as chronic atrophic gastritis (Sipponen et al., 2002), Zollinger-Ellison syndrome with multiple endocrine neoplasia type 1 (Zhong et al., 2005), pernicious anemia (Kedika, 2009) and conditions after surgery for vagotomy (Waldum et al., 2005). Hypergastrinemia is also a consequence of long-term antisecretory drugs administration (Mokrasch & Teschke, 1984; Tazoe et al., 2008).

Widespread infection of *Helicobacter pylori* also contributes to the increase in the percentage of people with hypergastrinemia (LeBlanc & LeBlanc, 2014). Another, equally important, consequence of the prolonged decrease in the secretion of hydrochloric acid (HCl) in the stomach is the development of dysbacteriosis in various biotopes of the digestive tract. Gastric secretion acid denatures proteins, activates pepsinogen and enhances absorption of iron and calcium in the intestine. However, according to Howden and Hunt (Hütt et al., 2009), the main function of gastric juice is the inactivation of ingested microorganisms. It is known that decrease in gastric juice acidity for any reason can lead to excessive bacterial growth in the oral cavity, the stomach (Habig et al., 1974; Wat-

son et al., 2002; Willams & McColl, 2006, 2018; Pylypenko et al., 2018) and in the small and large intestine (Habig et al., 1974; Osefo et al., 2009).

Proton pump inhibitors (PPI) are widely used to treat disorders caused by the action of hydrochloric acid: gastroesophageal reflux disease (GERD), Barrett's esophagus, peptic ulcer, functional and unresearched dyspepsia, Zollinger-Ellison syndrome, and other less common acid-dependent diseases. Proton pump inhibitors are a compulsory component of eradication therapy for *H. pylori* infection and are used for the prevention and treatment of NSAID-gastropathy (Radchuk et al., 2010; Tsyryuk et al., 2011). Acid-dependent diseases often require long-term drug treatment, and patients are constantly receiving PPI, which in its turn inhibits the protective effect of hydrochloric acid, leading to hypergastrinemia and dysbiosis. Therefore, hypergastrinemia and dysbiosis can develop as a result of long-term antisecretory drug (proton pump inhibitors) administration (Osefo, 2009). Both prolonged hypergastrinemia and dysbacteriosis enhance the development of chronic inflammatory process, which plays a decisive role in the development of tumours. The purpose of our project was to study the activity of antioxidant defense enzymes in the blood serum and in the colon mucosa of rats under the condition of prolonged omeprazole alone and omeprazole combined with the Symbiter and Apibact multiprobitotics administration.

Materials and methods

The work was performed on sexually mature white adult rats, which were kept under standard vivarium ration. Throughout the experiment, the animals were not abused and inhumanely killed. The research meets the basic requirements of keeping and working with laboratory animals in compliance with the rules of the European Convention for the Protection of Vertebrate Animals Used in Experimental Research and Other Scientific Purposes (Strasbourg, 1986) and in accordance with ethical standards specified in Ukrainian law (Murzin, 2004).

The conclusion that the experimental studies complied with the generally accepted bioethical standards and the relevant international regulations was made at the meeting of the bioethical committee of the National Institute of Biology of the National Taras Shevchenko University of Ukraine (26.06.2013, No. 35). The studies were performed on 40 white nonlinear male rats weighing 160–180 g, which were kept in an accredited vivarium at the Institute of Biology of Taras Shevchenko National University of Kyiv in compliance with the “Standard Rules for Organizing, Equipping and Maintaining Experimental Biological Clinics (Vivarium)”. All experiments were carried out in compliance with the Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelty”.

All animals were divided into 4 experimental groups. Animals of group 1 served as control. They were administered intraperitoneally (i/p) 0.2 mL and orally (p/o) 0.5 mL of water by injection for 28 days once a day. Animals of group 2 were administered omeprazole and 0.5 mL water for injection once a day for 28 days. Animals of group 3 were co-administered the Symbiter[®] acidophilic concentrated multiprobiotic (Simbiter) with omeprazole once a day for 28 days. In group 4, omeprazole and “Apibact[®]” multiprobiotic were co-administered once a day for 28 days.

Omeprazole (manufactured by “Sigma-Aldrich”, USA) was administered at a dose of 14 mg/kg dissolved in 0.2 mL of water for injection. Multiprobiotics Symbiter and Apibact (manufactured by NVK “OD Prolisok”, Ukraine) were co-administered with omeprazole p/o at a dose of 140 mg/kg ($1.4 \cdot 10^{10}$ CFU/kg). Multiprobiotics were dissolved in 0.5 mL of water for injection.

Activity of superoxide dismutase (CF 1.15.1.1) in cells was determined by Cevari et al. (1985). The method is based on the ability of superoxide dismutase to compete with nitro blue tetrazolium (NBT) for superoxide anions formed as a result of the aerobic interaction of the nicotinamide adenine dinucleotide reduced form and phenazin metasulfate (FMS). As a result of this reaction, NBT is restored to form hydrazine tetrazolium. In the presence of superoxide dismutase, the percentage of NBT recovery decreases.

Serum aliquote, cell lysate, or mucous membrane homogenate (0.5 mg of protein) was added to a sample containing 0.15 M phosphate buffer and the total sample volume was 0.5 mL. The amount of 1 mL reagent I (57 μ M HCT, 16 μ M FMS in 0.15 M phosphate buffer with EDTA, pH = 7.8) was added to the sample. Absorbance of the samples was immediately measured at $\lambda = 540$ nm with a spectrophotometer (SF-46, LOMO, Russia). Then, 0.035 mL of reagent II (98.5 μ M NAD.H in Tris-EDTA buffer, pH = 8.0) was added to each sample, placed in the dark and the extinction was re-determined after 10 min under the same conditions. The samples were kept at 30 °C. The enzyme activity was expressed in units per minute per 1 mg of protein.

The catalase activity (EC 1.11.1.6) in cells was determined by (Korolyuk et al., 1988). The principle of the method is that catalase destroys the H₂O₂ substrate, the non-destroyed part of hydrogen peroxide, when interacting with molybdenum salts, forms a stable coloured complex. 2 mL of 0.03% hydrogen peroxide solution were added to the test tubes. The reaction was started by adding 0.1 mL of serum, cell lysate, or mucous membrane homogenate (0.1 mg of protein). 0.1 mL of distilled water was added to the control sample instead of protein. The samples were kept at room temperature for 10 min, the reaction was stopped by adding 1 mL of 4% ammonium molybdate. The colour intensity was measured with a spectrophotometer (SF-46, LOMO, Russia) at $\lambda = 410$ nm against a control sample, which was added to 2 mL of H₂O instead of hydrogen peroxide.

Determination of the reduced glutathione content is based on modifications of the Koch and Lyle method (Howden & Hunt, 1987; Mikelsaar & Zilmer, 2009).

The method is based on the interaction of reduced glutathione with ortho-phthalic aldehyde (ORT) as a fluorescent reagent. This produces high-fluorescence products that are activated at 350 nm and have a well-pronounced peak at 420 nm. The ORT reagent was prepared before the experiment at a concentration of 1 mg/mL in methanol (Mikelsaar & Zilmer, 2009). It is shown that the reaction depends on the acid-alkaline characteristics of the medium, and takes place at pH 8.0, since a change in pH below the specified level is accompanied by a decrease in the fluorescence intensity, and conversely, the change of pH towards the alkaline medium causes the conversion of the reduced glutathione form into the oxidized one. The fluorescence intensity for ORT-GSH reaction directly and linearly depends on the concentration of GSH within the range of 10 ng to 4 μ g GSH.

The samples were thawed and homogenized on ice in a small Teflon Potter-Elvehjem homogenizer with addition of 1.5 mL of 0.01 M HCOH to precipitate proteins (Binder, 2010). Next, it was centrifuged in cold (+4 °C) at 20,000 g for 15 min to obtain a supernatant, in which the content of reduced glutathione was determined.

Sampling for GSH concentration was carried out according to the following scheme. To 0.1 mL of supernatant 100 μ L of 0.1 M phosphate buffer was added with (1:4 (V/V) 37% formalin (Tkachenko, 2014): 0.1 M Na₂HPO₄·12H₂O) (the samples were kept for 5 min at room temperature); 2 mL of 0.1 M phosphate buffer containing 1 mM Na₂HPO₄·12H₂O, 1 mM NaH₂PO₄·2H₂O, pH 8; 100 μ L of orthophthalic aldehyde. After the 45-minute incubation at room temperature, the fluorescence intensity at 420 nm was measured with 350 nm activation with a spectrofluorophotometer (RF-1501, Shimadzu, Japan). A calibration curve was constructed to determine the GSH concentration. GSH content was expressed in nmol per 1 mg of protein.

To determine the activity of glutathione-dependent enzymes, the serum, cell suspension, mucous membrane homogenate were centrifuged in cold (+4 °C) at 20,000 g for 15 minutes to obtain a supernatant, which was used to determine the enzymes activity.

The glutathione peroxidase activity (CE 1.11.1.9), as well as other glutathione-dependent enzymes, was determined by Vlasova (Vlasova & Perslegina, 1990). This enzyme activity was determined by accumulation of GSSG. The reaction mixture consisted of: 1 mL of 0.3 M phosphate buffer containing 12 mm sodium azide; 6 mm EDTA, pH 7.4; 0.5 mL of 2.5 mM GSSG; 0.2 mL of supernatant; 0.5 mL of 1.8 mM H₂O₂. The reaction was started by adding 0.5 mL of 1.8 mM hydrogen peroxide, stopped after 2 min by adding 0.5 mL of 10% trichloroacetic acid. After centrifugation at 3000 rpm for 15 min, the extinction of GSSG was determined at a wavelength of 260 nm. The enzyme activity was expressed in GSSG U-mol (mM) per 1 mg of protein per min by the formula: $A = a - b / c \times t$, where: a – the amount of oxidized glutathione in the control sample, taking into account non-enzymatic oxidation (nmol/mL); b – amount of oxidized glutathione in the test sample, taking into account non-enzymatic oxidation (nmol/mL); c – amount of protein in the test sample (mg); t – incubation time (min).

Glutathione transferase activity (CE 2.5.1.18) in cytosol was determined by the rate of the conjugate formation with 1-chloro-2,4-dinitrobenzene (CDNB), characterized by a maximum absorption at 346 nm (Hissin & Hilf, 1976; Vlasova & Perslegina, 1990).

To determine the glutathione transferase activity, a mixture was prepared: 1.5 mL of 0.1 phosphate buffer (pH 6.5), 0.2 mL of 10 mM GSH, 0.1 mL of the test sample supernatant. The reaction was started by adding 0.02 mL of 0.1 M CDNB. The increase in optical density was recorded for 3–5 min at 340 nm with a spectrophotometer (SF-6305, Lomo, Russia) and expressed in nanomoles (nM) of glutathione conjugate (GSR) per 1 mg of protein per min.

Glutathione reductase activity (CE 1.8.1.7) was determined by Vlasova et al. (1990), with modification for microcuvette measurement, with a total reaction mixture volume of 535 μ L. Determination of the enzyme activity was performed by reducing the NADPH content. The reaction mixture consisted of 350 μ L of phosphate buffer (0.05 M pH 8.0), 35 μ L of 1 mM EDTA, 50 μ L of 7.5 mM GSSG; 50 μ L of parietal cells cytosolic fraction; 50 μ L of 1.2 mM NADPH. The enzyme activity was determined by reducing the amount of NADPH at 37 °C for 8 min, at a wavelength of 340 nm with a spectrophotometer (SF-

6305, Lomo, Russia). The activity was expressed in NADPH nanomoles (nM) per 1 mg of protein per min.

Statistical data processing was performed in the Statistica 8.0 (StatSoft Inc., USA, 2009) software package. To test the samples for the type of distribution of the studied index the Shapiro-Wilk W-test was used. If the distribution of these samples did not correspond to the Gaussian distribution, then a nonparametric method was used to compare the samples – the Mann-Whitney U-test was applied for the comparison of two independent samples. In this case, the data obtained were presented as Median. The differences between the values in the control and experimental groups were determined using the ANOVA, where the differences were considered significant at $P < 0.05$ (with Bonferroni correction). The results were defined as means \pm standard error ($\bar{x} \pm SE$).

Results

After 28 days of omeprazole administration, the activity of superoxide dismutase (SOD) in the blood serum, which eliminates the superoxide anion radical due to its dismutation into hydrogen peroxide, decreased by 54.5% ($P < 0.05$) compared to the control (Fig. 1a). The activity of catalase increased by 211.5% ($P < 0.001$) (Fig. 1b).

With co-administration of omeprazole and the multiprobiotic, Symbiter SOD activity in the blood serum increased by 60% ($P < 0.05$) compared to the group of rats that received only omeprazole during the same time (Fig. 1a). At the same time, SOD activity remained 27.3% ($P < 0.05$) lower than in the control group. With co-administration of omeprazole and the multiprobiotic "Apibact", the blood serum SOD activity increased by 80% ($P < 0.05$) compared to the group of rats that received only omeprazole for 28 days and was statistically significantly different from the control group (Fig. 1a). In the experiments with co-administration of omeprazole and the multiprobiotic Symbiter and omeprazole and the multiprobiotic "Apibact", catalase activity in the rat blood serum decreased by 31.1% ($P < 0.05$) and 10.7% ($P > 0.05$), respectively, compared to the group of rats that received only omeprazole during the same time (Fig. 1b). However, this index remained significantly higher than in the control group (by 114.5% ($P < 0.05$) and 178.2% ($P < 0.01$), respectively). Activity of SOD and catalase in the mucous membrane of the rat colon after 28 days of omeprazole administration decreased by 35.9% ($P < 0.001$) and 45.6% ($P < 0.05$), respectively, compared to the control (Fig. 1a, b).

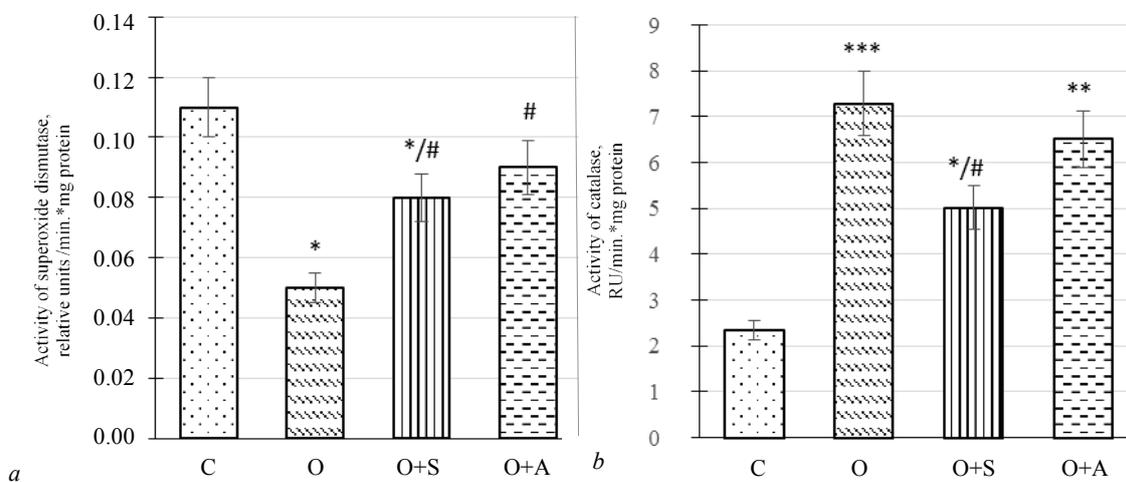


Fig. 1. Activity of superoxide dismutase (a) and catalase (b) in the blood serum of rats ($\bar{x} \pm SE$): C – the control group (n = 10); O – group of rats after 28 days of omeprazole administration (n = 10); O + S – group of rats after 28 days of omeprazole and "Symbiter" multiprobiotic co-administration (n = 10); O + A – group of rats after 28 days of omeprazole and "Apibact" multiprobiotic co-administration (n = 10); * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ compared to the control; # – $p < 0.05$ compared to the group of animals administered omeprazole

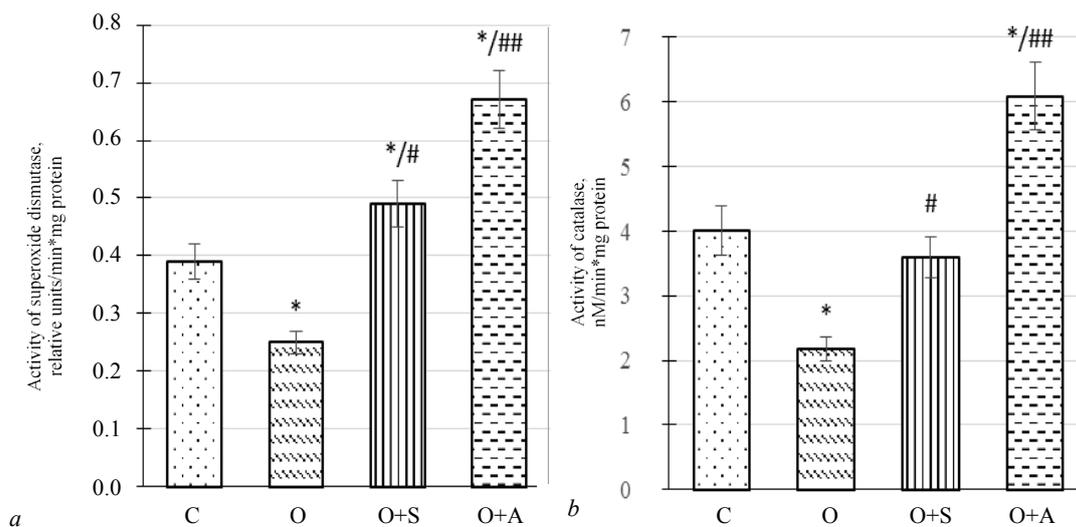


Fig. 2. Activity of superoxide dismutase (a) and catalase (b) in the mucous membrane of the rat colon ($\bar{x} \pm SE$): C – control group (n = 10); O – group of rats after 28 days of omeprazole administration (n = 10); O + S – group of rats after 28 days of omeprazole and multiprobiotic "Symbiter" co-administration (n = 10); O + A – group of rats after 28 days of omeprazole and multiprobiotic "Apibact" co-administration (n = 10); * – $P < 0.05$ compared to the control, # – $P < 0.05$, ## – $P < 0.01$ compared to the group of animals administered omeprazole

In the mucosa of rats which were co-administered omeprazole and the multiprobiotic "Symbiter" and omeprazole with the multiprobiotic "Apibact" for 28 days, SOD activity increased by 96.0% ($P < 0.05$) and 168.0%, respectively ($P < 0.01$ compared to the group of rats administered omeprazole only, Fig. 1a). However, the activity of SOD in groups of rats, which for 28 days were coadministered omeprazole and multiprobiotics together, exceeded even the control values. SOD activity under the conditions of omeprazole and "Symbiter" multiprobiotic co-administration was higher by 23.6% ($P < 0.05$), and under the conditions of omeprazole and "Apibact" multiprobiotic co-administration higher by 71.8% ($P < 0.01$) compared to the control.

The activity of catalase in the colon mucous membrane after prolonged co-administration of omeprazole and multiprobiotics also increased: after the omeprazole and multiprobiotic "Symbiter" co-administration it increased by 64.7% ($P < 0.05$) compared to the group of rats which were administered omeprazole only and statistically did not reliably differ from the controls; after omeprazole and multiprobiotic "Apibact" co-administration, the catalase activity increased by 179.4% ($P < 0.05$) compared to the rats treated with omeprazole only, which by 51.9% ($P < 0.05$) exceeded this index in the control (Fig. 1b). An important role in the implementation of antiradical and antiperoxide protection of cells is played by

the glutathione system, which consists of reduced glutathione and a set of enzymes – glutathione peroxidase, glutathione transferase and glutathione reductase. The components of the glutathione link in the antioxidant protection inhibit most of the free radical reactions, ensure the non-radical reduction of lipohydroperoxides, inactivate various toxic substances and contribute to maintenance of antioxidant protection (Freitas et al., 2009). In this respect, our further experiments were aimed at studying the glutathione antioxidant defense system functioning in the blood serum and colon mucosa of rats with prolonged gastric hypochlorhydria and under the conditions of omeprazole and multiprobiotics co-administration.

A study of the reduced glutathione (VG) content in the blood serum of rats after 28 days of omeprazole administration showed its decrease by 15.8% ($P < 0.05$) compared to the control (Fig. 3a). This may be due to both increased consumption of glutathione by glutathione-dependent enzymes (glutathione peroxidase, glutathione transferase) and glutaredoxin, and by the direct oxidation or reduction with participation of glutathione by SH groups of proteins (Oktyabrsky & Smimova, 2007).

After 28 days of gastric juice hypoacidity in the blood serum, glutathione peroxidase activity increased by 40.0% ($P < 0.05$, Fig. 3b), glutathione transferase activity increased by 31.6% ($P < 0.05$, Fig. 4a) and glutathione reductase activity decreased by 14.3% ($P < 0.05$, Fig. 4b).

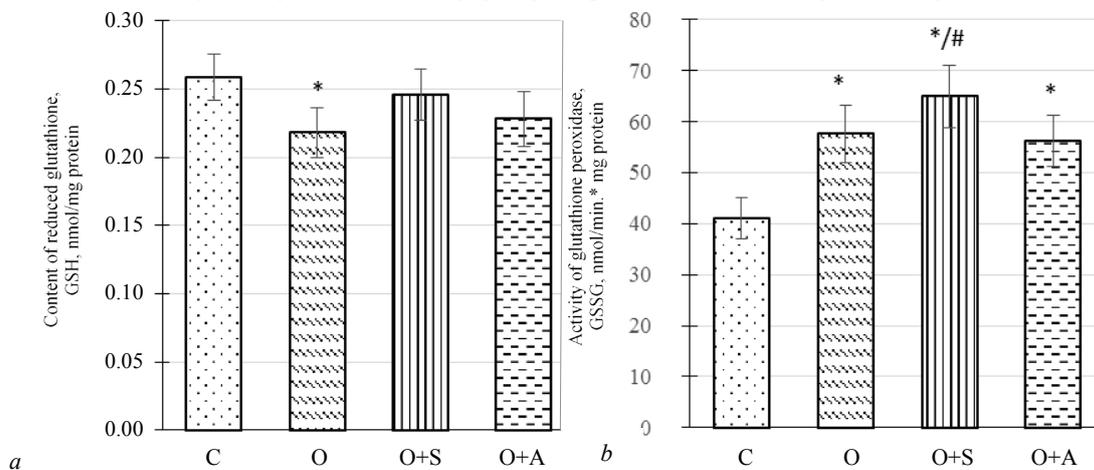


Fig. 3. The content ($x \pm SE$) of reduced glutathione (a) and activity of rat serum glutathione peroxidase (b): C – the control group ($n = 10$); O – group of rats after 28 days of omeprazole administration ($n = 10$); O + S – group of rats after 28 days of omeprazole and "Symbiter" multiprobiotic co-administration ($n = 10$); O + A – group of rats after 28 days of omeprazole and "Apibact" multiprobiotic co-administration ($n = 10$); * – $P < 0.05$ compared to the control; # – $P < 0.05$ compared to the group of animals administered omeprazole

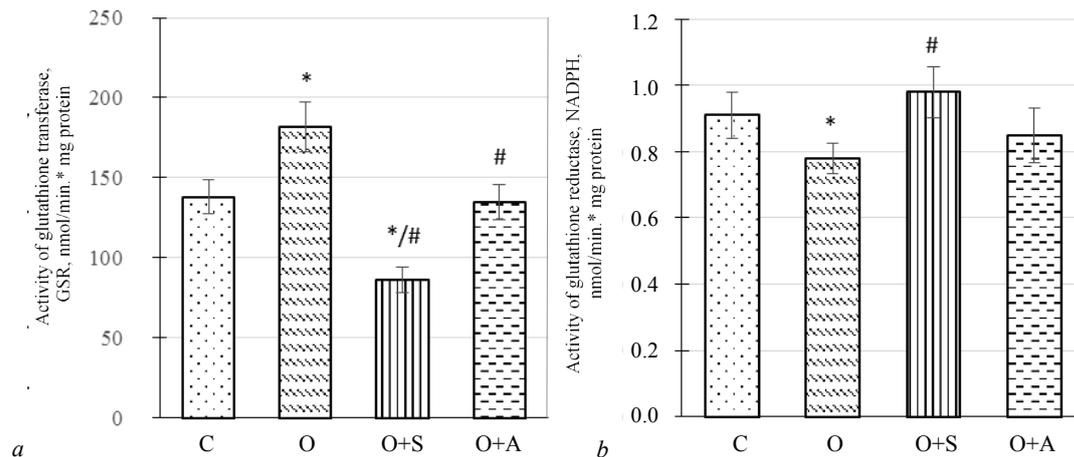


Fig. 4. Activity ($x \pm SE$) of glutathione transferase (a) and glutathione reductase (b) in the blood serum of rats: C – the control group ($n = 10$); O – group of rats after 28 days of omeprazole administration ($n = 10$); O + S – group of rats after 28 days of omeprazole and "Symbiter" multiprobiotic co-administration ($n = 10$); O + A – group of rats after 28 days of omeprazole and "Apibact" multiprobiotic co-administration ($n = 10$); * – $P < 0.05$ compared to the control; # – $P < 0.05$ compared to the group of animals administered omeprazole

Under conditions of 28-day co-administration of omeprazole and "Symbiter" or "Apibact" multiprobiotics, the serum RG content remained at the control level (Fig. 3a). Activity of glutathione peroxidase increased and exceeded the control values in the group of rats co-administered omeprazole and "Symbiter" multiprobiotic by 57.8% ($P < 0.05$), and in the group of rats co-administered omeprazole and Apibact multiprobiotic – by 36.8% ($P < 0.05$, Fig. 3b). Activity of glutathione transferase in groups of rats co-administered omeprazole and "Symbiter" multiprobiotic and omeprazole and "Apibact" multiprobiotic was higher by 31.6% ($P < 0.05$) and 31.6% ($P < 0.05$) respectively compared to the control (Fig. 4a).

Activity of glutathione reductase in groups of rats co-administered omeprazole and "Symbiter" multiprobiotic and omeprazole and "Apibact" multiprobiotic was lower by 14.3% ($P < 0.05$) and 14.3% ($P < 0.05$) respectively compared to the control (Fig. 4b).

prazole and “Apibact” multiprobiotic for 28 days decreased by 52.7% ($P < 0.05$) and 25.9% ($P < 0.05$), respectively, compared to the group of rats, which were administered omeprazole only (Fig. 4a).

In this case, the glutathione transferase activity in the blood serum in the group of rats administered omeprazole and “Apibact” multiprobiotic, reached the control values (Fig. 4a). The activity of glutathione reductase in the blood serum of rats co-administered omeprazole and multiprobiotics increased and was not statistically reliably different from the similar values in the control group (Fig. 4b).

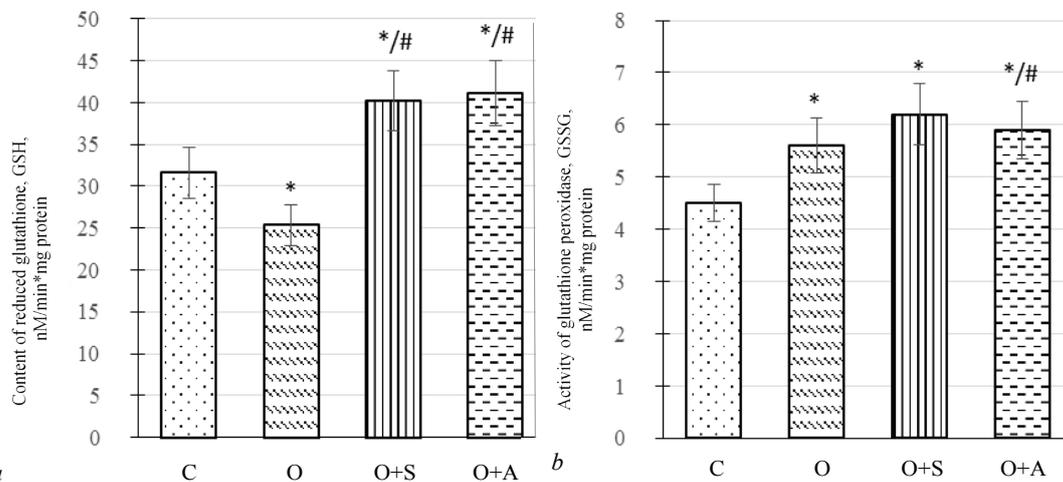


Fig. 5. The content of reduced glutathione (a) and the activity of glutathione peroxidase (b) in the mucous membrane of the rat colon ($x \pm SE$): C – the control group ($n = 10$); O – group of rats after 28 days of omeprazole administration ($n = 10$); O + S – group of rats after 28 days of omeprazole and “Symbiter” multiprobiotic co-administration ($n = 10$); O + A – group of rats after 28 days of omeprazole and “Apibact” multiprobiotic co-administration ($n = 10$); * – $P < 0.05$ compared to the control; # – $P < 0.05$ compared to the group of animals administered omeprazole

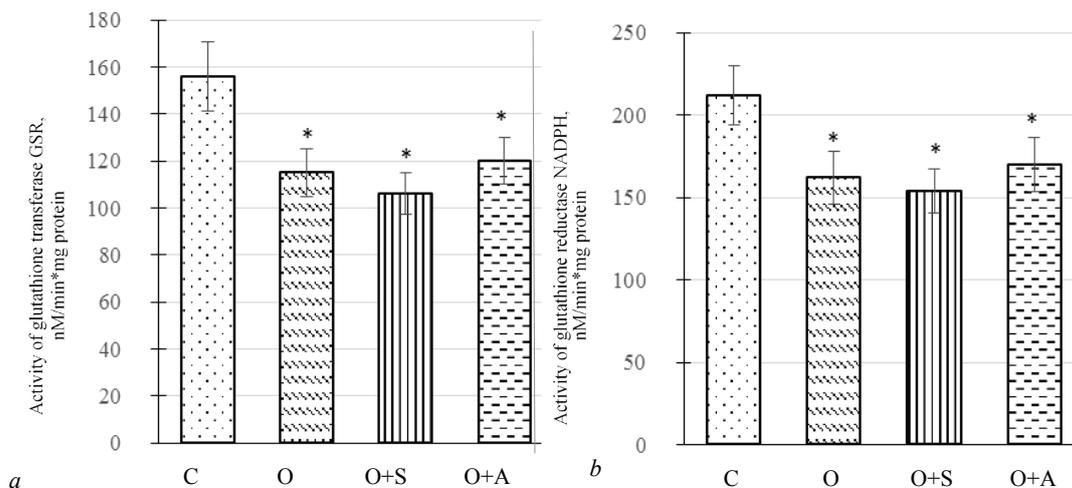


Fig. 6. The activity of glutathione transferase (a) and the activity of glutathione reductase (b) in the mucous membrane of the rat colon ($x \pm SE$): C – the control group ($n = 10$); O – group of rats after 28 days of omeprazole administration ($n = 10$); O + S – group of rats after 28 days of omeprazole and “Symbiter” multiprobiotic co-administration ($n = 10$); O + A – group of rats after 28 days of omeprazole and “Apibact” multiprobiotic co-administration ($n = 10$); * – $P < 0.05$ compared to the control

After the 28-day co-administration of omeprazole with “Symbiter” multiprobiotic and omeprazole with “Apibact” multiprobiotic to rats, the content of reduced glutathione in the colon mucosa increased by 58.3% ($P < 0.05$) and 61.8% ($P < 0.05$), respectively, in comparison with the group of rats which were administered omeprazole only; by 27.2% ($P < 0.05$) and by 30.1% ($P < 0.05$) exceeding this index compared to the control (Fig. 4a). The activity of glutathione peroxidase (Fig. 4b), glutathione transferase (Fig. 5a) and glutathione reductase (Fig. 5b) in the mucous membrane of the rat colon after 28 days of omeprazole and multiprobiotics co-administration did not undergo significant changes compared to the similar indices in the group of rats which were administered omeprazole only.

Discussion

After 28 days of omeprazole administration, the reduced glutathione (RG) content in the colon mucosa decreased and was by 19.6% ($P < 0.05$) lower than in the control group (Fig. 5a). In this group of rats, glutathione peroxidase activity increased by 24.4% ($P < 0.05$, Fig. 5b), and glutathione transferase and glutathione reductase activity decreased by 26.3% ($P < 0.05$, Fig. 5a) and 23.6% ($P < 0.05$, Fig. 5b), respectively, compared to the control.

Therefore, we can assert that under the conditions of prolonged gastric hypoacidity, depletion of SOD in the serum occurs as a result of excessive synthesis of reactive oxygen forms by phagocytic cells in development of the digestive tract inflammation, induced by dysbiosis and hypergastrinemia. SOD activity can also be reduced due to its inactivation by hydrogen peroxide, which is accumulated in the blood serum. The evidence of hydrogen peroxide accumulation is growth of catalase activity in the blood serum (Fig. 1b). There are reports that omeprazole inactivates one of the SOD isoforms, which may also cause the reduction in SOD activity (Elchuri & Oberley, 2005). Finally, there may be one more reason for the decrease in SOD enzymatic activity in these conditions, namely: the oxidative modification of histidine residues that bind copper and zinc in the active center of the SOD molecule (Hosokawa et al., 1985).

Under the action of free radicals, including hydrogen peroxide, the residue of His-118 is oxidized to 2-oxyhistidine. Other histidine residues under the influence of free radicals are converted into aspartate or asparagine (Liochev & Fridowich, 2002). With regard to the increase in the content of H_2O_2 in the blood serum, H_2O_2 can be formed, first, in the spontaneous dismutation of the superoxide radical, the content of which is increased as a result of the SOD activity reduction. Secondly, hydrogen peroxide is formed during the reaction of the superoxide radical with the peroxide radical. Therefore, the increase in catalase activity may be a consequence of the activated bypass pathways in hydrogen peroxide formation (Ozbakis et al., 2007). The literature analysis indicates that the obtained data on the antioxidant properties of multiprobiotics are expected. After all, the phenomenon of the LPO process inhibition by different strains of lactic acid bacteria is described in a number of works (Hütt et al., 2009). In addition, it has been shown that the Apibact multiprobiotic reduces the content of LPO products in the pancreas and liver of rats under prolonged gastric hypoacidity induced by omeprazole (Dvorshchenko & Ostapchenko, et al., 2010). Significant changes in the activity of antioxidant enzymes in the rat mucosa were observed after prolonged administration of omeprazole. Increase in the content of TBA-reactive substances in the mucous membrane of the colon indicates an impairment of the prooxidant-antioxidant balance, and testifies to the development of inflammation in this organ (Pellicano et al., 2006; Pylypenko & Koval, 2018; Pylypenko et al., 2018).

In the colon, oxidative / nitrosative stress, which was caused by hypergastrinemia and dysbacteriosis and was accompanied by an increase in the TBA-reactive substances and NO production, is also the result of the SOD enzymes and antioxidant protection system's catalase depletion, which may be due to accumulation of free-radical compounds in the mucous membranes, affecting the intestinal mucosa and causing the inflammatory process in the intestine. The results suggest that after prolonged inhibition of HCl secretion in the stomach, the glutathione antioxidant defense link is depleted in the colon mucosa, as a result of the over-activation of free radical reactions and LPO, and the cells cannot resist the oxidative stress against the background of inflammatory process induced by hypergastrinemia and dysbiosis. Oxidative stress is important for removal of pathogenic microorganisms, although overproduction of reactive oxygen forms or impaired endogenous antioxidant protection can lead to disastrous effects on the cells and tissues of the host organism (Friis-Hansen, 2006; Boyko et al., 2016). The cause of glutathione depletion in the antioxidant system may also be due to the direct action of omeprazole, which is metabolized with the participation of glutathione (Zavros & Merchant, 2005). We believe that the work that revealed the antioxidant properties of omeprazole (Burdan et al., 2001; Biswas et al., 2003; Kaur et al., 2009) cannot be compared with our results, since the authors studied the effects of short-term use of proton pump inhibitors in the treatment of gastroduodenal ulcer.

Therefore, intensification of LPO reactions and NO generation in the balance disorders in the functioning of the antioxidant protection enzymes and depletion of the glutathione link of the antioxidant system indicate a decrease in the antioxidant potential of the colon mucosa against the background of prolonged gastric hypochlorhydria. Prolonged co-administration of omeprazole and multiprobiotics led to a significant normalization of the antioxidant system's enzyme activity, including its glutathione link. Reasons for intensification of LPO reactions in the large intestinal mucosa under the conditions of the prolonged HCl secretion inhibition in the stomach may be, on the one hand, insufficient activity of antioxidant enzymes, on the other, increased generation of reactive oxygen forms and NO. Indeed, it has been proved that the inducer of free radical lipid oxidation in dysbacteriosis is an excess of reactive oxygen forms and nitrogen generated by phagocytes.

Suppression of hydrochloric acid secretion in the stomach leads to dysbacteriosis throughout the digestive tract, which only intensifies over time. In the colon dysbiosis, the balance between obligate, optional and transient microflora is significantly impaired. In the development of dysbiosis, the concentration of obligate representatives of anaerobic sucralitic flora (bifidobacteria, lactobacilli, etc.) decreases first of all, and against this background there is an increase in the level of opportunistic

microorganism populations (*Escherichia*, enterococci, clostridia) and an increase in their aggressive potential (Makarchuk et al., 2019). This is accompanied by a decrease in the physiological activity of the microbiome, which are expressed in the development of various bowel dysfunctions and in the emergence of local and systemic pathological processes. Accumulation of the opportunistic microflora to the "critical level", accompanied by the natural activation of its virulent properties, can lead to the development of serious pathological disorders. Downturn of dysbiosis is associated with disorders of metabolic, morphokinetic, functional, and sometimes structural changes in the colon, with the activation of lipid peroxidation, antioxidant defense suppression, decrease of reticuloendothelial system activity, and immune processes, decrease of the liver detoxication function, synthesis of vitamins, enzymes, mediators, short-chain fatty acids, accumulation of toxic compounds. Simultaneously, the processes of fermentation and nutrient uptake, in which the biofilm indigenous microbiota takes an active part are decreasing, physiological regeneration of colonocytes is suppressed, intercellular membranes are damaged, the tissue antigens are formed, permeability of the gastrointestinal tract is increased with development of alimentary allergy (Yankovskyy et al., 2017).

Almost all acute and chronic diseases of the digestive system, as well as other organs and systems, are accompanied by intestinal dysbiosis of varying severity (Kharchenko & Anokhina, 2005). Therefore, to ensure complete clinical cure, a compulsory component of complex therapy for patients with any gastrointestinal disease, as well as a number of other diseases that depend on the condition of the digestive tract, should include the correction of microecological disorders. To do this, probiotics are used created on the basis of living cells of lactobacilli and bifidobacteria (Sanders, 2011; Koch et al., 2012).

The modulating effect of multiprobiotics on the processes of free radical oxidation in the colon mucosa under the conditions of inflammatory process development is obviously the result of the elimination of oxygen metabolism disorders in phagocytes and protection of mucous membranes from aggressive action of reactive oxygen and nitrogen forms. The literature analysis shows that the obtained data on the antioxidant properties of multiprobiotics are expected, and the phenomenon of the LPO process inhibition by different strains of lactic acid bacteria is described (Forman et al., 2009). In addition, the "Apibact" multiprobiotic has been shown to reduce the content of LPO products in the pancreas and liver of rats under the conditions of long-term gastric juice hypoacidity caused by omeprazole (Dvorshchenko & Ostapchenko, 2010).

Consequently, multiprobiotics activate the antioxidant protection enzymes in the mucous membrane of the colon by reducing the intensity of free radical lipid oxidation, normalize the quantitative and qualitative composition of microflora, reduce gastrin levels in the blood serum and directly neutralize reactive oxygen forms and H_2O_2 and activate other links of the antioxidative defense (Metz, 2012).

Conclusions

Prolonged gastric juice hypochlorhydria led to changes in the functioning and depletion of antioxidant protection enzymes: compared to the control, the blood serum SOD activity decreased by 54.5% ($P < 0.05$) and the catalase activity increased by 211.5% ($P < 0.05$). Prolonged gastric juice hypochlorhydria led to changes in the functioning and depletion of antioxidant defense enzymes: compared to the controls in the colon mucosa, SOD and catalase activity decreased by 35.9% ($P < 0.001$) and 45.6% ($P < 0.05$). Under the conditions of prolonged gastric hypochlorhydria, the functioning of the glutathione antioxidant protection system was impaired in the colon mucosa. Under the conditions of prolonged gastric hypochlorhydria, functioning of the glutathione antioxidant defense system was impaired in the colon mucosa. Prolonged administration of multiprobiotic drugs against the background of gastric hypochlorhydria significantly reduced the inflammatory process manifestations in the mucous membrane of the large intestine, which was expressed in the normalization of the antioxidant system enzymes activity, including its glutathione link.

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